University of São Paulo
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Movement ecology and functional relationships in natural enemies of *Spodoptera frugiperda*

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Thesis presented to obtain the degree of Doctor in Science. Area: Entomology

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Dedication

I dedicate this thesis to my beloved parents, Genilda and Audimair, for all their support and love.

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“But ask the animals, and they will teach you,
or the birds in the sky, and they will tell you;
or speak to the earth, and it will teach you,
or let the fish in the sea inform you.
Which of all these does not know that the hand of the Lord has done this?
In his hand is the life of every creature and the breath of all mankind.”

Job 12:7-10

RESUMO

Ecologia do movimento e relações funcionais em inimigos naturais de *Spodoptera frugiperda*

Estudar as nuances de um agroecossistema para que programas de manejo de pragas envolvendo a combinação de plantas transgênicas e inimigos naturais seja sustentável ambientalmente e economicamente é um grande desafio. Dessa forma, esta pesquisa teve como objetivo estudar a interação de práticas de manejo sobre os inimigos naturais de *Spodoptera frugiperda* (Lepidoptera: Noctuidae), considerando um cenário agrícola composto de plantas Bt (*Bacillus thuringiensis*) e diferentes genótipos da praga. Conduzimos um estudo para investigar, primeiramente, se diferentes genótipos interagiriam diferentemente com agentes de controle biológico, abordando sistemas predador-presa específicos para a espécie em questão. Realizamos experimentos de predação e resposta funcional em laboratório com ninfas e adultos do percevejo predador *Podisus nigrispinus* (Hemiptera: Pentatomidae), sobre larvas de *S. frugiperda* resistentes e suscetíveis às toxinas Bt. Adultos e ninfas apresentaram resposta funcional do tipo II, independente do genótipo da presa. A dinâmica de predação também foi a mesma para ambos os genótipos, no entanto, a predação máxima foi atingida por adultos diante de presas suscetíveis. Ninfas levaram ~2.9 minutos para se alimentar das larvas. A análise de PCA mostrou que o comportamento de predação dos predadores depende do genótipo da presa. A nossa pesquisa também investigou como a resistência em *S. frugiperda* ocorre em áreas de milho Bt e não-Bt, arranjadas espacialmente em faixas e de forma aleatória, cercadas de plantas gramíneas (*Cynodon* sp.), considerando períodos de entressafra. Utilizamos modelagem computacional baseada no indivíduo para criar a paisagem proposta e inserimos os dados biológicos da praga junto ao modelo. Os resultados mostraram que a presença de gramíneas contribuiu na diminuição do alelo de resistência em campo. Arranjos aleatórios foram favoráveis ao aumento de indivíduos resistentes no campo (95.86%). Arranjos em faixas mostraram ser mais eficientes no manejo da resistência, mas ainda assim possibilitaram um aumento de 82.10%. Períodos de entressafra contribuíram tanto para a redução da população de *S. frugiperda,* quanto para a redução da frequência do alelo de resistência. Por fim, ainda utilizando modelagem computacional baseada no indivíduo, estudamos a combinação de técnicas de manejo de pragas: plantas Bt, não-Bt, inimigos naturais de *S. frugiperda* e inseticidas. Nosso objetivo foi entender como essas ferramentas atuam no manejo da resistência e de que maneira essa integração poderia afetar os inimigos naturais (parasitoides) e, consequentemente, seus serviços ecossistêmicos. Os resultados mostraram que zonas de refúgio maiores beneficiam os inimigos naturais e, ao mesmo tempo, auxiliam na redução da frequência de resistência em campo. Por outro lado, essas áreas favoreceram o aumento da população de pragas em campo. Inseticidas com 50 e 100% de seletividade contribuíram com a permanência de parasitoides, juntamente com zonas de refúgios nas proporções de 15 e 20%. Os cenários com menor zona de refúgios (5%) e seletividade de 0% sobre os inimigos naturais, tiveram aumento na frequência de resistência inicial (1%) e, portanto, foram consideradas inapropriados para o manejo de pragas.

Palavras-chave: Plantas Bt, Modelagem computacional, Inimigos naturais, Resistência de pragas

ABSTRACT

Movement ecology and functional relationships in natural enemies of *Spodoptera frugiperda*

Studying the nuances of an agroecosystem so that pest management programs involving the combination of transgenic plants and natural enemies are environmentally and economically sustainable is a great challenge. Therefore, this research aimed to study the interaction of management practices on the natural enemies of *Spodoptera frugiperda* (Lepidoptera: Noctuidae), considering an agricultural scenario composed of Bt plants (*Bacillus thuringiensis*) and different genotypes of the pest. We conducted a study to investigate, firstly, whether different genotypes would interact differently with biological control agents, addressing specific predator-prey systems for the species in question. We carried out predation and functional response experiments in the laboratory with nymphs and adults of the predatory bug *Podisus nigrispinus* (Hemiptera: Pentatomidae), on larvae of *S. frugiperda* resistant and susceptible to Bt toxins. Adults and nymphs showed a type II functional response, regardless of prey genotype. The predation dynamics was the same for both genotypes, however, the maximum predation was reached by adults facing susceptible prey. Nymphs spent ~2.9 minutes to feed on larvae. The PCA analysis showed that the predation behavior of predators depends on the prey genotype. Our research also investigated how resistance in S. frugiperda occurs in areas of Bt and non-Bt maize, spatially arranged in bands and randomly, surrounded by grassy plants (*Cynodon nlemfuensis*), considering off-season periods. We used individual-based computational modeling to create the proposed landscape and entered the biological data of the pest along with the model. The results showed that the presence of grasses contributed to the reduction of the resistance allele in the field. Random (seed mixture) arrangements were favorable to the increase of resistant individuals in the field (95.86%). Arrangements in strips proved to be more efficient in managing resistance, but still allowed an increase of 82.10%. Off-season periods contributed both to the reduction of the population of *S. frugiperda* and to the reduction in the frequency of the resistance allele. Finally, still using computational modeling based on the individual, we studied the combination of pest management techniques: Bt and non-Bt plants, natural enemies of *S. frugiperda* and insecticides. Our goal was to understand how these tools act in resistance management and how this integration could affect natural enemies (parasitoids) and, consequently, their ecosystem services. The results showed that larger refuge zones benefit natural enemies and, at the same time, help to reduce the frequency of resistance in the field. On the other hand, these areas favored an increase in the population of pests in the field. Insecticides with 50 and 100% selectivity contributed to the permanence of parasitoids, along with refuge zones in proportions of 15 and 20%. The scenarios with the smallest refuge zone (5%) and 0% selectivity on natural enemies, had an increase in the frequency of initial resistance (1%) and, therefore, were considered inappropriate for pest management.

Keywords: Bt crops, Computational modeling, Natural enemies, Insect resistance

1. GENERAL INTRODUCTION

The great demand for food has increasingly required innovative solutions regarding pest management, taking environmental sustainability into account. The challenge is to identify which is the best pest control measure to be used, or a set of them. The IPM (Integrated Pest Management) is the global term defined by FAO (2012) as the combined use of techniques that aim to increase agricultural production in a healthy way, reducing the application of pesticides. The IPM system does not despise conservative control and values ecological interactions, such as predation and parasitism as control agents as much as the use of GM (Genetically Modified) crops and pesticides (Lundgren et al. 2009).

In this context, several questions have emerged: Is there compatibility between GM crops and natural enemies? How to study these interactions? So far, research has shown that the use of GM crops (plants expressing the insecticidal toxin from soil bacterium *Bacillus thuringiensis* - Bt) is highly effective and very specific to insect pests (Head, 2005). However, when friendly organisms are strongly related to the target pests of the controlling tactic, which will be potential risks, whether chemical or biological control (Dale et al., 2002).

Spodoptera frugiperda (Lepidoptera: Noctuidae), is the main target of Bt technologies around the world. Due to its high management cost and the increasing cases of insecticide resistance, Bt toxins were the great control alternative for the pest. Meantime, a few years after the widespread introduction of Bt technology, field-evolved resistance emerged requiring new pest management techniques (Huang, 2021), and valuing even more the natural control by natural enemies, which can keep these pests below the level of economic damage (Liu et al., 2014; Koffi et al., 2020).

Some studies have investigated how this tritrophic relationship among Bt crops-herbivory-natural enemies occurs focusing on behavioral traits of these insects, such as feeding, oviposition, and movement of phytophagous (Ramalho et al., 2014; Han et al., 2016). Most works have investigated, indirectly, whether the performance of natural enemies can be impaired when the prey or host has been feeding on transgenic plants (Schuler et al., 2004; Malaquias et al., 2014; Meissle et al., 2005). These studies involve predation and behavioral experiments.

However, regarding to the movement of pests, which can be a challenge for effective IPM strategies (Irwin, 1999), modeling has proved to be helpful to guide efficient pest management techniques (Malaquias et al., 2017). Models can be used as a

predictive way, since they can investigate the factors that affects the population dynamics of pest and identify which are the essential factors to stabilize ecological interactions between natural enemies and pests (Tang and Cheke, 2008).

Faced with the questions raised related to pest management, Bt crops, ecological traits, natural enemies and its prey/host, our first objective was to study the dynamics of predation of the natural enemy *Podisus nigrispinus* (Hemiptera: Pentatomidae) as well as its functional response over different genotypes of *S. frugiperda*. Resistance to Bt toxins has been documented in many parts of the world (Vélez et al., 2013; Omoto et al. 2016). Nonetheless, few studies have focused on exploring resistance in the context of natural control by predators (Romeis et al 2006; Malaquias et al., 2015).

The second stage of the research was developing a model based on individual, using cellular automata approaches, to investigate the insect movement in crops field, structured with Bt and non-Bt maize. Furthermore, we inserted a naturally occurring herbaceous component in Florida - USA, which could or could not contribute to the evolution of pest resistance in *S. frugiperda.*

In the third phase of the thesis, we study how integrating of biological control techniques, Bt crops, and refuge zones (non-Bt) could affect the resistance evolution in *S. frugiperda.* Nonetheless, aiming to bring our model closer to the practice in the field when integrated management is applied, we insert the effects of a hypothetical insecticide over natural enemies to show whether the natural control is negatively affected.

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